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The goal of our research is to use molecular and genetic techniques to investigate the process of bacterial attachment to and colonization of surfaces in the marine environment. We are focussing on exploring the mechanism which controls surface-induced swarmer cell differentiation of *Vibrio parahaemolyticus*. Gene fusions which couple transcription of swarmer cell genes, *laf*, to luminescence reporter genes, *lux*, have been used to analyze how environmental signals regulate differentiation, and a novel mechanism of surface recognition involving a tactile sensor and an iron sensor has been discovered. Work is continuing to develop a refined understanding of the sensors and other elements in the information transduction circuit which controls expression of swarmer cell genes.

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RESEARCH OBJECTIVE: Our goal is to understand the molecular mechanisms which control microbial colonization of surfaces in the marine environment. We have been focusing on the surface-induced differentiation of *V. parahaemolyticus* because exploration of this adaptive response could reveal a mechanism for recognition of surface contact.

PROGRESS (YEAR 1 AND 2): *V. parahaemolyticus* has two distinct cell types, the swimmer cell and the swarmer cell, which are adapted for survival in different habitats. The swimmer cell, produced when the bacterium is grown in a liquid medium, is a short rod with a single sheathed polar flagellum. The swarmer cell, produced when *V. parahaemolyticus* is grown in contact with surfaces, is greatly elongated and synthesizes, in addition to the polar flagellum, numerous lateral flagella which are responsible for translocation over surfaces. Differentiation to the swarmer cell appears to be an appropriate adaptation for life on surfaces because cells with lateral flagella adhere more firmly to surfaces and swarming expands the area of colonization on the surfaces. But, how does the bacterium recognize contact with a surface and activate the genetic program (*laf* genes) encoding the swarmer cell phenotype?

The transcription of genes encoding the swarmer cell phenotype has been analyzed with *laf::lux* gene fusion strains constructed in *V. parahaemolyticus* with transposon mini-Mu*lux*. This approach simplified examination of gene regulation since light emission, encoded by the *lux* genes, was measured instead of complex morphological and behavioral events. Gene fusion strains were used to show that swarmer cell genes were induced by a variety of conditions, including a high viscosity environment or antibody cross-linking, which had in common the constraint of movement of the polar flagellum. To test the hypothesis that the polar flagellum is functioning as a tactile sensor which controls swarmer cell formation, we constructed a variety of mutations in genes encoding components of the polar flagellum (*fla*). The consequence of such mutations was the constitutive, surface-independent, expression of *laf* genes. So, the performance of the polar flagellum (Fla) is coupled to

the transcription of the *laf* genes such that when Fla function is purturbed, either physically or genetically, swarmer cell genes are induced. Because the polar flagellum appears to be capable of sensing external forces influencing its motion, we suggest it is operating as a dynamometer.

Another environmental input has been found to influence swarmer cell formation. In addition to stimulation of the tactile sensor (the polar flagellum), a second signal, iron limitation, is required for swarmer cell differentiation. Differentiation requires a large investment of cellular resources, and by basing the "decision" to differentiate on multiple inputs an appropriate response to a specific environmental condition could best be accomplished. We have also found that the polar (Fla) and lateral (Laf) flagellar systems show behavioral coupling. The two appendages are assembled from different motor-propellar components, but chemotactic control of the behavior of swimmer and swarmer cells is controlled by one common information processing apparatus.

WORK PLAN (YEAR 3): We have identified signals which induce swarmer cell differentiation and have discovered that the polar flagellum functions as a tactile sensor controlling differentiation. Research will now focus on understanding how this sensor works at the molecular level. The polar flagellum can be expected to be very complex with many components involved in the assembly of the motor-propellar structure, in the energy transduction machinery driving propeller rotation, in the chemosensory system directing flagellar movement in response to environmental stimuli and also in the tactile sensor function. We will use mutants to attempt to separate tactile sensor function from the behavioral response function of the polar flagellum and to determine what component or specific flagellar activity is directly involved in controlling expression of the swarmer cell phenotype.

We are particularly interested in analyzing chemotaxis mutants. These can be constructed by localized mutagenesis of the cloned *che* genes and subsequent transfer of mutations to *V. parahaemolyticus* by a gene replacement procedure. It is known from analysis of paralyzed (Mot$^-$) mutants that flagellar rotation is required for tactile sensing, and with Che$^-$ mutants it should be possible to determine if chemosensory function is also necessary. And, Che$^-$ mutants are usually locked into either a clockwise or counter-clockwise rotational mode so the influence of the direction of propeller rotation on sensing can be examined. Another approach is to search for genes whose products directly regulate the expression of swarmer cell genes. Swarmer cell genes, *laf*, appear to be regulated by negative rather than by positive control of transcription since mutants with defects in the tactile sensor are constitutive for *laf* expression rather than being uninducible which would be the consequence of a defect in a positive effector of transcription. We will search for genes encoding a repressor of *laf* transcription by programming expression of cloned genes positioned on an expression vector *in trans* in *V. parahaemolyticus*.

PUBLICATIONS FROM THIS PROJECT:

1. McCarter, L. and M. Silverman. 1987. Phosphate regulation of gene expression in *V. parahaemolyticus*. J. Bacteriol. **169**:341-3449.

2. Belas, R., Bartlett, D. and M. Silverman. 1988. Cloning and gene replacement mutagenesis of a *Pseudomonas atlantica* agarase gene. Appl. and Env. Microbial. **54**:30-37.

3. McCarter, L., Hilmen, and M. Silverman. 1988. Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. Cell. **54**:345-351.

4. Simon, M., Belas, R., McCarter, L., Bartlett, D., and M. Silverman. 1988. Microbial role in biodeterioration: Advanced Techniques Applicable to the Indian Ocean, Oxford and IBH Publishing, New Delhi, India, Eds. American Institute of Biological Sciences, p. 265.

5. McCarter, L. and M. Silverman. 1989. Iron regulation of swarmer cell differentiation of *V. parahaemolyticus*. J. Bacteriol. **171**:731-736.

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